

A novel PCV2 qPCR and PCV2 ELISA. Powerful tools to Monitor PCVAD

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Introduction

Detecting and quantifying Porcine Circovirus type 2 (PCV2) in both clinically and sub-clinically (PCV2 virus-) infected pigs is important. PCV2 virus, even in low quantities, has a detrimental effect on their performance. A lower Average Daily Weight Gain (ADWG) and a less efficient Feed Conversion Ratio (FCR) are mainly held responsible for the economic damage¹. To monitor PCV2 Associated Disease (PCVAD) both PCV2 qPCR and ELISA tests have been developed. Virus neutralizing (VN) antibodies are detected by both IPMA and ELISA and they control PCV2 viremia². PCV2 viremia is monitored by qPCR. Both test kits are developed and produced by BioChek and tested for their respective suitability.

Materials and methods

The PCV2 ELISA test is typically used to determine the presence and titer of maternally derived antibodies (MDA), sero-conversion after vaccination or field virus infection, to check the uniformity of immunity and to determine the right vaccination moment. The PCV2 qPCR is used for checking for presence and quantity (viral load) of PCV2 virus. The BioChek PCV2 ELISA test was compared to other tests present in the market and to the IPMA test, which is the gold standard for detecting PCV2 antibodies, and use was made of well-defined serum samples³.

The BioChek PCV2 qPCR participated in a Proficiency Testing Scheme (PTS) or ring test, organized by the Animal Health Service (AHS) (GD) organization, Deventer, the Netherlands. In this PTS, 8 different freeze dried samples were included and divided in 3 groups. The group with sample numbers 1, 5 and 7 contained PCV2 field virus strains in different dilutions. The group with sample numbers 2 and 4 did not contain any PCV2 virus (negative or PCV type 1 virus). The group with sample numbers 3, 6 and 8 contained a different PCV2 virus strain in several dilutions. The 8 different samples were tested at the BioChek BV laboratories in Reeuwijk, the Netherlands; using the BioChek PCV2 qPCR test kit from a routinely produced and released batch.

As part of the development of the BioChek PCV2 qPCR, serial dilutions of PCV2 virus were tested. The BioChek PCV2 qPCR contains a positive standard to help diagnostic laboratories check their internal procedures.

Results

The BioChek PCV2 ELISA showed an excellent correlation with the IPMA test. This was tested and reported by Pileri³.

The PTS testing for the BioChek qPCR revealed that the result of every individual test was in line with the result of the PTS leader. Sample numbers 2 and 4 (no PCV2 virus) scored negative in the BioChek PCV2 qPCR. Confirming the declared specificity of >99%. PCV2 virus positive samples (1, 5 and 7)

scored positive in the BioChek PCV2 qPCR. Number 5 was highly diluted; sample 7 contained slightly less PCV2 virus than number

1. This was reflected in the Cq values, 5 had the highest Cq value, while 7 was just higher than 1. For the PCV2 virus positive sample numbers 3, 6 and 8 a similar pattern was found: High dilution - high Cq value. Proving high sensitivity (high dilution and still positive) and good linearity.

The PCV2 virus serial dilutions testing revealed the same level of linearity as was seen in PTS testing and a sensitivity of as low as 2.8 on a log₁₀ scale in viral copies per ml serum⁴, which is even more sensitive than a regular PCV2 PCR. Also the good linearity between the samples in different dilutions can be observed (figure 1). In figure 2 the results of testing different batches of the PCV2 standard included in the BioChek qPCR test kit are shown. Excellent similarity between the samples originating from different batches of positive controls was found.

Discussion

PCVAD can effectively be controlled by vaccination. The vaccination efficiency depends on the immunity induced by the vaccine. VN antibodies are the 1st line of defense. When no sero-conversion after vaccination is observed and PCV2 viremia is detected, the vaccination scheme should be reconsidered. The goal of PCV2 vaccination is to raise pigs that do not suffer from PCVAD. For monitoring the efficiency of PCV vaccination the combined information on seroconversion obtained by ELISA and PCV2 viremia by qPCR is needed. The financial gains can be significant⁵.

References

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